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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/538,305	06/09/2005	Kenji Miyazaki	Q88457	3672

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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/538,305	MIYAZAKI ET AL.	
	Examiner	Art Unit	
	ROBERT XU	1797	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 June 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-19* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *09 June 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/9/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Summary

1. This is the first Office action based on the 10/538,305 application filed on June 9, 2005.

2. Claims 1-19 are pending and have been fully considered.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

5. **Claims 1-11** are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita et al. (Electrophoresis, 1998) (Tsugita) in view of Tsugita et al.'1992 (Chemistry Letters, 1992) (Tsugita'1992) and Covey et al. (US Patent No. 5,952,653) (Covey).

In regard to Claim 1, Tsugita teaches a method of analyzing the C-terminal amino acid sequence of a peptide. The method comprises the following steps:

a step of preparing a mixture containing a series of reaction product by chemically releasing the C-terminal amino acids successively (see page 930, right col. 3rd paragraph);

a step of analyzing the differences in molecular weight between series of reaction products and the original peptide by means of mass spectrometry and identifying a series of the amino acid removed successively based on a series of the measured decreases in molecular weight (see page 931, left col. 2nd paragraph).

The step of preparing the mixture further comprises the following sub-steps:

a pretreatment sub-step for providing the protection of N-terminal and lysine side chain by reacting a protein with an 20% acid anhydride in tetrahydrofuran at 60° for 10 min; the N-terminal of the protein is protected by acetylation and an amino acid residue at C-terminal is modified to generate oxazolone (see page 930, right col., 3rd paragraph); and

a cleavage sub-step for allowing oxazolone of the N-terminal protected protein or peptide to react with 5% pentafluoropropionic methyl ester (PFPMe) in methanol at 5° for 15 min to successively release the C-terminal amino acid (see page 930, right col., 3rd paragraph). Tsugita uses PFPMe in the cleavage sub-step in the procedure (see page 930, right col., 3rd paragraph), while claim 1 recites perfluoroalkanoic acid (PFPA). Tsugita uses PFPA for cleavage at C-side of aspartic acid and the N-side of serine/threonine and for simultaneous successive truncation at the C-termini of the

cleaved fragments (see abstract). Tsugita'1992 teaches using PFPA in the cleavage sub-step of the procedure to successively release N-terminal amino acid (see page 236, flow chart). PFPMe and PFPA have similar structure and functions. At the time of the invention, it would have been obvious to ordinary skill in the art to use PFPA in the cleavage sub-step of Tsugita's procedure based on Tsugita'1992 teaching.

Tsugita further teaches that 20% acetic anhydride is used in the pretreatment sub-step for applying N-acetylation protection to the N-terminal of the protein and for forming oxazolone at C-terminal of the protein, and 5% PFPMe is used in the cleavage sub-step to react with oxazolone (page 930, right col. 3rd paragraph). Tsugita does not specifically teach maintaining acetic anhydride in the cleavage sub-step. However, since the function of acetic anhydride is to form oxazolone at C-terminal for perfluoroalkanoic acid to act on in the cleavage sub-step, it would have been obvious to ordinary skill in the art to recognize that maintaining the concentration of acetic anhydride in the cleavage sub-step may benefit the reaction.

Tsugita further teaches a hydrolysis treatment sub-step, for allowing the reaction product to react with an amine to hydrolyze the ester (see page 930, right col., 3rd paragraph).

The analyzing step further comprises the following sub-steps:

a sub-step of analyzing the result by MALDI-TOF-MS or FAB-MS (see page 931, left col. 2nd paragraph);

a sub-step of identifying a series of the amino acids based on a series of the decreases in molecular weight (page 936, Table 3).

Tsuguta also teaches that the routine measurement of protease-cleaved fragments have been used for identification, referred to as "peptide-mass fingerprinting" (see page 929, left col. 4th paragraph). Tsuguta further demonstrates a sub-step of cleavage at carboxyl side of aspartic peptide bond (Asp-C) and the amino side of the serine or threonine peptide bonds (Ser/Thr-N) before mass spectrometry analysis to improve the accuracy because of reduced peptide size (see page 931, left col. last paragraph; right col. 1st paragraph). However, Tsuguta is silent on using trypsin to cleave the carboxyl side of arginine peptide bond (Arg-C) to improve the accuracy of mass spectrometry analysis.

Covey discloses a method of using trypsin to cleave protein and analyzing the result by mass spectrometry (see abstract). Covey teaches that arginine is basic and picks up a positive charge proton in solution. Thus, the tryptic fragments will be doubly positively charged because of the inclusion of arginine at C-terminal and an amino terminus in each fragment (see Col. 2, lines 63-66). Covey further teaches that the charge difference makes the C-terminus fragment to stand out from the other tryptic fragments, because the C-terminus fragment has no arginine at C-terminus and therefore, will not have two positive charges (see col. 5, lines 58-62). In other words, in the mass spectra, the C-terminus fragment shows stronger intensity in anionic species, and all the other tryptic fragments show stronger intensity in cationic species. At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin cleavage and mass spectra analysis method as taught by Covey in the second step of Tsugita with reasonable expectation, that trypsin cleavage would therefore

increase the accuracy of the mass spectrometry measurement, because the trypsin cleavage reduces the size of peptide for mass spectrometry analysis, and in the mass spectra, the C-terminal peptide fragments obtained by successive release of the C-terminal amino acids would stand out by showing stronger intensity in anionic species, while all the other tryptic fragments would show stronger intensity in cationic species, as specifically indicated by Covey.

In regard to Claims 2-4, the modified method of Tsugita utilizes acetic anhydride as the alkanoic acid anhydride contained in a mixture of an alkanoic acid anhydride with a small amount of a perfluoroalkanoic acid added (see page 930, right col. 3rd paragraph).

In regard to Claim 5, the modified method of Tsugita uses PFPA as the perfluoro-alkanoic acid. The pH of PFPA is 0.8.

In regard to Claims 6 and 7, PFPA, used by Tsugita'1992 in successive releasing C-terminal amino acid, has 3 carbon atoms linear-chain.

In regard to Claim 8, as has been discussed in regard to Claim 1, in light of teachings of Tsugita and Tsugita'1992, the ratio of PFPA (5%) to acetic anhydride (20%) would be 1:4. In the instant Claim, the lower limit is 20:100 or 1:5. The Applicants are advised that generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ

233, 235 (CCPA 1955). Therefore, it would have been obvious to one of ordinary skill in the art to discover the optimum ratio of perfluoroalkanoic acid to alkanoic acid anhydride by a routine experimentation.

In regard to Claims 9 and 10, Tsugita teaches that the pretreatment and cleavage sub-steps needs to be carried out in the absence of water (page 931, right col. 2nd paragraph, last 2 lines; page 930, 3rd paragraph). Tsugita'1992 teaches that the target peptide is dried in a small test tube. The tube is placed in a large test tube which contained PFPA in acetonitrile. The large tube is flame sealed under vacuum (page 235, last paragraph). This implies that the reaction is carried out in an air-tight container, where the inside oxygen has been eliminated.

In regard to Claim 11, the temperature of 5ºC used in the cleavage sub- step of Tsugita's procedure is lower than the range of 15 ºC to 50ºC recited in the instant claim. Applicant is advised that generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Therefore, it would have been obvious to one of ordinary skill in the art to discover the optimum range of the reaction temperature by routine experimentation.

6. **Claims 12-19** are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Tsugita'1992, Vogt et al. (Polymer Bulletin, 1996) (Vogt) and Covey.

In regard to Claim 12, Tsugita teaches a method of analyzing the C-terminal amino acid sequence of a peptide. The method comprises the following steps:

a step of preparing a mixture containing a series of reaction product by chemically releasing the C-terminal amino acids successively (see page 930, right col. 3rd paragraph);

a step of analyzing the differences in molecular weight between series of reaction products and the original peptide by means of mass spectrometry and identifying a series of the amino acid removed successively based on a series of the measured decreases in molecular weight (see page 931, left col. 2nd paragraph).

The step of preparing the mixture further comprises the following sub-steps:

a pretreatment sub-step for providing the protection of N-terminal and lysine side chain by reacting a protein with an 20% acid anhydride in tetrahydrofuran at 60° for 10 min; the N-terminal of the protein is protected by acetylation and an amino acid residue at C-terminal is modified to generate oxazolone (see page 930, right col., 3rd paragraph);

a cleavage sub-step for allowing oxazolone of the N-terminal protected protein or peptide to react with 5% penta-fluoropropionic methyl ester (PFPMe) in methanol at 5° for 15 min to successively release the C-terminal amino acid (see page 930, right col., 3rd paragraph); Tsugita uses PFPMe in the cleavage sub-step in the procedure (see page 930, right col., 3rd paragraph), while claim 12 recites perfluoroalkanoic acid (PFPA). Tsugita uses PFPA for cleavage at C-side of aspartic acid and the N-side of serine/threonine and for simultaneous successive truncation at the C-termini of the

cleaved fragments (see abstract). Tsugita'1992 teaches using PFPA in the cleavage sub-step of the procedure to successively release N-terminal amino acid (see page 236, flow chart). PFPMe and PFPA have similar structure and functions. At the time of the invention, it would have been obvious to ordinary skill in the art to use PFPA in the cleavage sub-step of the procedure based on teaching of Tsugita'1992 and Tsugita.

Tsugita teaches that 20% acetic anhydride is used in the pretreatment sub-step for applying N-acetylation protection to the N-terminal of the protein and for forming oxazolone at C-terminal of the protein, and 5% PFPMe is used in the cleavage sub-step to react with oxazolone (page 930, right col. 3rd paragraph). Tsugita does not specifically teach maintaining acetic anhydride in the cleavage sub-step. However, since the function of acetic anhydride is to form oxazolone at C-terminal for perfluoroalkanoic acid to act on in the cleavage sub-step, it would have be obvious to ordinary skill in the art to recognized that maintaining the concentration of acetic anhydride in the cleavage sub-step may benefit the reaction.

Tsugita further teaches a hydrolysis treatment sub-step, for allowing the reaction product to react with an amine to hydrolyze the ester (see page 930, right col., 3rd paragraph);

Tsugita does not teach using polar aprotic solvent to remove water. However, using polar aprotic solvent to remove water is known in the art. Also, Tsugita does not teach using dipolar-aprotic solvent to swollen the gel so that the pretreatment and cleavage sub-step reaction could be carried out on the protein bound to the original gel right after electrophoresis. Tsugita teaches that the pretreatment and cleavage sub-

step of the procedure needs to be carried out in the absence of water (page 931, right col. 2nd paragraph, last 2 lines; page 930, 3rd paragraph). Therefore, the target protein has to be extracted from the gel and then dried to remove water or electroblotted to an Immobilon-CD membrane.

Vogt teaches a new non-aqueous swelling system; specifically he teaches that carboxymethyl cellulose (CMC) gel treated with a dipolar aprotic solvent like *N,N*-dimethylacetamide with *p*-toluenesulfonic acid yields a high reactive gel-suspension of the polymer (see abstract). This dipolar aprotic solvent can remove water from the swollen gel in one step (see page 550, 3rd paragraph), thus allowing a direct esterification of the hydroxyl group of CMC (see abstract). At the time of the invention, it would have been obvious to one of ordinary skill in the art to use polar aprotic solvent to remove water and use dipolar aprotic solvent to remove water from the gel carrier bound with the target protein, as taught by Vogt with reasonable expectation that this would allow Tsugita's procedure to be carried out on the target protein kept on the gel carrier.

The analyzing step further comprises the following sub-steps:

a sub-step of analyzing the result by MALDI-TOF-MS or FAB-MS (see page 931, left col. 2nd paragraph).

a sub-step of identifying a series of the amino acids based on a serious of the decreases in molecular weight (page 936, Table 3).

Tsuguta also teaches that the routine measurement of protease-cleaved fragments have been used for identification, referred to as "peptide-mass fingerprinting"

(see page 929, left col. 4th paragraph). Tsuguta further demonstrates a sub-step of cleavage at carboxyl side of aspartic peptide bond (Asp-C) and the amino side of the serine or threonine peptide bonds (Ser/Thr-N) before mass spectrometry analysis to improve the accuracy because of reduced peptide size (see page 931, left col. last paragraph; right col. 1st paragraph). However, Tsuguta is silent on using trypsin to cleave the carboxyl side of arginine peptide bond (Arg-C) to improve the accuracy of mass spectrometry analysis.

Covey discloses a method of using trypsin to cleave a protein and analyzing the result by mass spectrometry (see abstract). Covey teaches that arginine is basic and picks up a positive charge proton in a solution. Thus, the tryptic fragments will be doubly positively charged because of the inclusion of arginine at C-terminal and an amino terminus in each fragment (see Col. 2, lines 63-66). Covey further teaches that the charge difference makes the C-terminus fragment stands out from the other tryptic fragments, because the C-terminus fragment has no arginine at C-terminus and therefore, will not have two positive charges (see col. 5, lines 58-62). In other words, in the mass spectra, the C-terminus fragment shows stronger intensity in anionic species, and all the other tryptic fragments show stronger intensity in cationic species. At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin cleavage and mass spectra analysis method as taught by Covey in the second step of Tsugita with reasonable expectation, that trypsin cleavage would therefore increase the accuracy of the mass spectrometry measurement, because the trypsin cleavage reduces the size of peptide for mass spectrometry analysis, and in the mass

spectra, the C-terminal peptide fragments obtained by successive release of the C-terminal amino acids would stand out by showing stronger intensity in anionic species, while all the other tryptic fragments would show stronger intensity in cationic species.

In regard to Claims 13-15, the modified method of Tsugita utilizes acetic anhydride as the alkanoic acid anhydride contained in a mixture of an alkanoic acid anhydride with a small amount of a perfluoroalkanoic acid added (see page 930, right col. 3rd paragraph).

In regard to Claim 16, the modified method of Tsugita utilizes PFPA as the perfluoroalkanoic acid. The pH of PFPA is 0.8.

In regard to Claims 17 and 18, PFPA, used by Tsugita'1992 in successive releasing C-terminal amino acid, has 3 carbon atoms linear-chain.

In regard to Claim 19, as has been discussed in regard to Claim 12, in light of teachings of Tsugita and Tsugita'1992, the ratio of PFPA (5%) to acetic anhydride (20%) would be 1:4. In the instant Claim, the lower limit is 20:100 or 1:5. The applicants are advised that generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Therefore, it would have been obvious to one of ordinary skill in the art to discover the optimum ratio of perfluoroalkanoic acid to alkanoic acid anhydride by routine experimentation.

Conclusion

7. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Tsugita et al. (US Patent No. 6,046,053) (Tsugita'2000) teach a method of sequencing protein by successively releasing C-terminal residue with treatment of acetic anhydride and PFPA followed by hydrolysis in DMAE solution (Col. 9, lines 10-40).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROBERT XU whose telephone number is (571)270-5560. The examiner can normally be reached on Mon-Thur 7:30am-5:00pm, Fri 7:30am-4:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill Warden can be reached on (571)272-1267. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Yelena G. Gakh/
Primary Examiner, Art Unit 1797

RX